1: J Immunol Methods 1995 Nov 16;187(1):23-31

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# Hydrogen peroxide destaining: a new method for removing non-specific stains in nitrocellulose membrane-based dot-ELISA for the detection of trypanosomes in tsetse flies (Glossina spp.).

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Gut samples prepared from laboratory-reared tsetse flies and applied in dots onto nitrocellulose (NC) membrane were found to stain the membrane with differing coloration and intensity. The stains were, predominantly, either reddish to brown or blackish-brown to black and occasionally greenish to almost colourless, depending on the stage of digestion of the bloodmeal in the fly. NC membrane strips applied with tsetse gut samples from T. brucei infected and uninfected control flies were tested with the standard antigen detection dot enzyme-linked immunoassay (dot-ELISA), using a T. brucei specific monoclonal antibody (MoAb) and horseradish peroxidase goat anti-mouse conjugate. The stains in both infected and uninfected sample dots persisted through the assay. Furthermore, the staining intensity of some assayed uninfected sample dots were enhanced as a result of non-specific reactivity, making it difficult to distinguish between the infected and uninfected flies. This necessitated the development of a simple technique by which the non-specific stains and reactions could be removed. Sample 'dotted' NC membrane strips were destained by incubation with 5% hydrogen peroxide (H2O2) diluted in 5% skimmed milk in Tris buffer, pH 8.0. After washing, the destained strips were tested in the dot-ELISA. This method gave satisfactory reproducible results, since the most intense stains could be removed, and it had no effect on trypanosome antigens detected by a panel of four T. brucei species-specific, three T. vivax species-specific, four T. congolense species-specific and four Nannomonas subgenus-specific MoAbs. Using the destaining process in a modified dot-ELISA, 86 out of 95 (90.5%) of Glossina morsitans centralis flies experimentally infected with T. brucei, were identified. The destaining method was also used successfully to decolorize NC membrane bound tsetse faecal material.

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